of the anhydrous and hydrated chromium orthophosphates under both stable and metastable equilibrium. The suggestion of Ness, Smith and Evans[3] that on heating the hexahydrate or the anhydrous phase above 200° an oxidation of the chromium occurs will need re-apprasial. If the formula be written as $CrPO_4 \cdot OH$ a suggestion of a higher oxidation state appears. On the other hand, it is unlikely that dehydration to a "semi-hydrate" will cause oxidation, especially in view of the fact that at higher temperatures the material reverts to the trivalent chromium in the orthophosphate, and at still higher temperatures forms Cr_2O_3. The evidence shows nothing which contradicts the formulation of this phase as the hydroxy phosphate of Cr(III).

The powder X-ray data showed that this phase had no relation to any silica forms, nor were any other metastable phases encountered which could be regarded as silica mineral analogs. It appears most unlikely that Cr^{3+} can accept a four-coördinated position with respect to oxygen and, hence, no chromo-silicate chemistry analogous to alumino-silicate chemistry can be developed since the basic property on which the latter depends is the substitution of Al^{3+} for Si^{4+} in tetrahedral coördination.

(3) A. T. Ness, R. E. Smith and R. L. Evans, THIS JOURNAL, **74**, 4685 (1952).

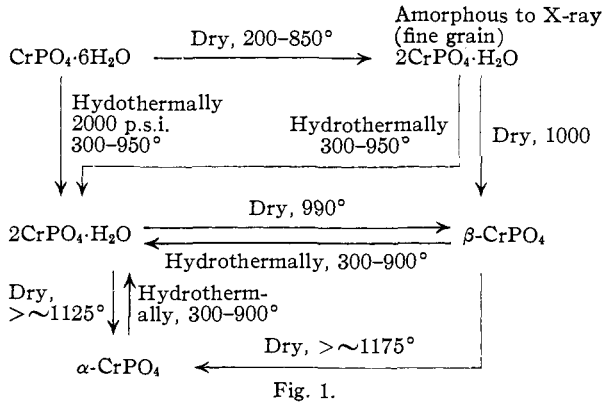

Fig. 1.

Acknowledgment.—We wish to acknowledge the assistance of Mr. J. J. Comer for his part in obtaining the electron diffraction patterns. This work was supported by the U.S. Army Signal Corps Engineering Laboratories, Project SC-5594.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BARNARD COLLEGE, COLUMBIA UNIVERSITY]

The Thermodynamics of Ionization of Amino Acids. II. The Ionization Constants of Some N-Acyl Amino Acids[1]

BY EDWARD J. KING AND GRACE W. KING

RECEIVED AUGUST 23, 1955

The thermodynamic ionization constants of some N-acyl amino acids were obtained from measurements of the electromotive forces of cells with hydrogen and silver–silver chloride electrodes which contained buffer mixtures of the weak acids, their sodium salts and sodium chloride. Measurements were made at 5° intervals between 5 and 50°, and the ionization constants were fitted to the equation $-\log K = (A/T) + B + CT$. The values of the parameters are as follows: *A:* 1248.54, 1101.03, 908.48, 906.43 and 1279.32; *B:* −4.8146, −3.7708, −2.8416, −2.9315 and −3.9494; *C:* 0.014411, 0.012730, 0.011771, 0.012096 and 0.013763 for N-acetylglycine, N-propionylglycine, N-acetyl-DL-alanine, N-acetyl-DL-α-amino-*n*-butyric acid and N-acetyl-β-alanine, respectively. The changes in free energy, entropy, enthalpy and heat capacity associated with the ionization reaction in the standard state as well as the properties at the temperature at which the ionization constant reaches its maximum value are compared with those of other carboxylic acids. The entropies of ionization of the acyl amino acids lie between those of the fatty acids and the α-amino acids. This is related in large part to the orientation of water about the peptide linkage. The entropy of ionization of acetic acid is more negative than that of formic acid because the order-producing methyl group in the former projects into the region of disordered water outside the primary hydration layer about the carboxylate ion. Smaller fluctuations in the entropy of ionization associated with chain branching and lengthening are attributed to the strengthening of the water structure by alkyl groups and to restricted internal rotation in the anions.

The N-acyl amino acids occupy a strategic position among the carboxylic acids. They are composed of neutral molecules (HA), as are the fatty acids, so that their ionization creates charged particles: $HA + H_2O \rightleftarrows H_3O^+ + A^-$. Their molecules contain the peptide linkage, –CONH–, found also in peptides and proteins. From that standpoint they are important model compounds to consider before any concerted attack is made on the ionization of peptides themselves. From the theoretical point of view the presence of the peptide linkage is interesting because it is polar, it will interact with water,[2] and it will act as a chain-stiffener[3,4] inasmuch as the internal rotation about the carbon–nitrogen bond is severely restricted.[5] The entropy of ionization may be expected on this basis to show some interesting effects. Work is currently in progress in this Laboratory on the ionization of peptides and also of some N-carbamoyl amino acids in which the terminal methyl group of the acetyl derivatives is replaced by the hydrophilic, polar amino group.

The ionization constants of the N-acetyl derivatives of glycine, DL-alanine, β-alanine and DL-α-amino-*n*-butyric acid and N-propionylglycine have

(1) This investigation was supported by a research grant, H-1651, from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

(2) E. F. Mellon, A. H. Korn and S. R. Hoover, THIS JOURNAL, **70**, 3040 (1948).

(3) D. H. Everett, D. A. Landsman and B. R. W. Pinsent, *Proc. Roy. Soc.* (*London*), **215A**, 403 (1952).

(4) A. G. Evans and S. D. Hamann, *Trans. Faraday Soc.*, **47**, 34 (1951).

(5) S. Mizushima, T. Simanouti, S. Nagakura, M. Kuratani, M. Tsuboi, H. Baba and O. Fujioka, THIS JOURNAL, **72**, 3490 (1950).

been obtained from measurements on cells of the type[6]

$$(Pt)H_2\,|HA(m_1),\ NaA(m_2),\ NaCl(m_3)\,|AgCl\text{-}Ag \quad (I)$$

Measurements were made at 5° intervals from 5 to 50° to permit calculation of heats and entropies of ionization and related quantities.

Experimental

Apparatus.—The hydrogen supply line was reconstructed largely of one-eighth inch copper tubing with Hoke toggle and metering valves, helium leak-tested, and Swagelok tube fittings. The laboratory barometer was cleaned and recalibrated by the manufacturer after the work on the first acid, acetylglycine. Other features of the apparatus have been described before.[7,8]

Materials.—Dilute hydrochloric acid and carbonate-free sodium hydroxide solution were made and standardized in the same way as in previous work.[9] Water used in making these solutions and all others was ordinary distilled water passed through a column of Amberlite MB-1 monobed ion-exchange resin. It had a specific conductance of about 0.3 gemmhos. The sodium chloride was the bromide-free sample described before.[7] Silver oxide for the silver–silver chloride electrodes was prepared by slow addition of carbonate-free sodium hydroxide solution to dilute silver nitrate solution. The precipitated oxide was washed repeatedly with water by decantation for a period of eleven days. The dry product was found by flame photometry to contain less than 0.00006% sodium.

The N-acetylglycine was a C.P. product (H. M. Chemical Co.) treated with washed Norite and recrystallized three times from water. The N-acetyl-DL-alanine was obtained from Mann Research Laboratories and used without further purification. The acetyl-α-amino-*n*-butyric acid was also from this source, but it was treated with Norite, recrystallized twice and air-dried. The N-propionylglycine was synthesized in our laboratory from propionic anhydride and glycine and was recrystallized from water and air-dried. The N-acetyl-β-alanine was supplied by Mann Research Laboratories as a thick sirup. After standing at room temperature for two weeks this crystallized to a yellowish cake smelling strongly of acetic acid. The compound was very soluble in polar solvents and its solutions supersaturated badly. It was crystallized from acetone to give white, odorless crystals, m.p. 78.3–80.3°.

All of the acids were assayed by titration with standard sodium hydroxide solution. Water was determined by the Karl Fischer method. Various semi-quantitative tests[10] also were made. These results are summarized in Table I. It will be seen that all of the acids are of satisfactory purity except the β-alanine derivative. Repeated recrystallization of this product gave no improvement in its assay. The product was examined by paper chromatography for free β-alanine and none was found although, under the conditions of the experiment, 0.1% could have been detected. Formol titration gave the same assay as that reported below. The product does not contain free amino acid. No evidence that the product was a mixture of acids could be obtained by paper or column chromatography.

TABLE I

Test	N-Acetyl-glycine	N-Acetyl-DL-alanine	N-Acetyl-DL-α-amino-*n*-butyric acid	N-Acetyl-β-alanine	N-Propionyl-glycine
Assay	99.5%	99.7%	100.0%	98.4%	99.8%
Water	0.38	0.02	0.04	0.26	0.11
Ash	.05	.....	.....	.....	.....
Fe	< .004	< .004	< .004	< .004	< .004
Cl	< .004	< .05	< .004	< .004	< .01
NH_3	< .004	< .004	< .004	[a]	< .004
PO_4	< .004	< .004	< .004	< .004	< .004
H.M.	< .004	< .004	< .004	< .004	< .004

[a] A satisfactory test for free NH_3 was not obtained because the compound gave a precipitate with Nessler reagent.

Anal. Calcd. for $C_6H_9NO_3$: C, 45.80; H, 6.92; N, 10.68. Found: C, 46.0; H, 6.5; N, 10.6.[11]

The performance of our apparatus and technique was checked by making measurements on the cells

$$H_2\,|HCl(m)\,|AgCl\text{-}Ag \quad (II)$$

One of us had reported before[12] that such cells in our laboratory gave higher electromotive forces than those of Harned and Ehlers[13] above 25°. Our present measurements confirm this and point to an explanation. The copper to mercury contacts of the leads to the hydrogen electrodes are outside the cells and above the water level of the constant temperature bath. The contacts at the silver–silver chloride electrodes are inside the cells. A thermal electromotive force results which makes the voltage of a cell too high above 25°, the maximum effect being 0.11 mv. at 50°, and too low below 25°, the maximum effect being −0.09 mv. at 5°. This is not sufficient to account for the whole of the discrepancy between our results and those of Harned and Ehlers. But the difference in standard potentials at 25 and 50° ($E^0_{25} - E^0_{50}$) = 0.01785 v., obtained by us from measurements on cell II is in good agreement with the recent determinations of Bates and Bower[14] (0.01785) and Harned and Paxton[15] (0.01790) and not with that of Harned and Ehlers (0.01802). We conclude that the temperature coefficient of our results, after correction for thermal electromotive force, is satisfactory. The E^0 value at 25°, 0.22258 abs. v., is considerably higher than that of Bates and Bower or Harned and Paxton though reasonably close to that of Harned and Ehlers. This high value may reflect differences in depth of introduction of hydrogen into the solution[16] or a decrease in the electromotive forces of the standard cells since their calibration.[17] It has seemed best to minimize these systematic errors by using working standard electromotive forces, E^0_W, consistent with our apparatus. These have been derived by increasing all of the results of Bates and Bower by 0.24 mv. so that E^0_W at 25° is equal to 0.22258 v., the average of our own results, and by including also the thermal electromotive forces. The values of E^0_W so obtained are: 5°, 0.23429; 10°, 0.23160; 15°, 0.22876; 20°, 0.22576; 25°, 0.22258; 30°, 0.21930; 35°, 0.21594; 40°, 0.21240; 45°, 0.20868; 50°, 0.20484 abs. v. These values agree with our own limited experimental results on cell II with a standard deviation of ±0.036 mv. They are used in all of the calculations that follow.

The general techniques used in the measurements on cell I have been described before.[7–9] Solutions were prepared from weighed quantities of acid, sodium hydroxide solution, sodium chloride and conductance water. The addition of the acid and sodium chloride to the sodium hydroxide and the final weighing were made just before filling the cells. The performance of the cells gives no evidence of hydrolysis of the peptide linkage. The standard deviations in mv. between initial and final readings at 25° taken about 30 hours apart were 0.053 for acetylglycine, 0.043 for acetyl-DL-alanine, 0.037 for acetyl-α-amino-*n*-butyric acid, 0.032 for acetyl-β-alanine and 0.050 for propionylglycine. The electromotive forces, corrected to a hydrogen pressure of one atmosphere, can be represented as a function of temperature by the equation

$$E_t = E_{25} + a(t - 25) + b(t - 25)^2 \quad (1)$$

The parameters of this equation for electromotive forces in absolute volts and concentrations in moles per kilogram of

(6) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd ed., Reinhold Publ. Corp., New York, N. Y., 1950, pp. 497–516.

(7) E. J. King, THIS JOURNAL, **73**, 155 (1951).

(8) E. J. King, *ibid.*, **74**, 1212 (1952).

(9) E. J. King, *ibid.*, **76**, 1006 (1954).

(10) M. P. Stoddard and M. S. Dunn, *J. Biol. Chem.*, **142**, 329 (1942).

(11) Analyses by Schwarzkopf Microanalytical Laboratory, Middle Village 79, N. Y.

(12) E. J. King, THIS JOURNAL, **75**, 2204 (1953).

(13) H. S. Harned and R. W. Ehlers, *ibid.*, **54**, 1350 (1932); **55**, 652, 2179 (1933).

(14) R. G. Bates and V. E. Bower, *J. Research Natl. Bur. Standards*, **53**, 283 (1954).

(15) H. S. Harned and T. R. Paxton, *J. Phys. Chem.*, **57**, 531 (1953).

(16) G. J. Hills and D. J. G. Ives, *J. Chem. Soc.*, 305 (1951).

(17) G. W. Vinal, "Primary Batteries," John Wiley and Sons, Inc., New York, N. Y., 1950, Chapter 6.

water are given in Table II. The standard deviations between the observed electromotive forces and those calculated from equation 1 are ±0.078 mv. for acetylglycine, 0.023 for propionylglycine, 0.025 for acetyl-DL-alanine, 0.024 for acetyl-DL-α-amino-*n*-butyric acid and 0.030 for acetyl-β-alanine.

TABLE II

PARAMETERS OF EQUATION 1

m_1	m_2	E_{25}	$a \times 10^5$	$b \times 10^8$
Acetylglycine, m_1/m_2 = 1.000				
0.00825	0.00836	0.56427	525	0
.02019	.02107	.53975	442	0
.03193	.03890	.52390	389	0
.04949	.05015	.51708	365	25
.07001	.07003	.50854	338	0
.10087	.10911	.49708	294	0
m_1/m_2 = 1.168				
0.01178	0.01004	0.55532	496	0
.02405	.02060	.53634	431	0
.03348	.02886	.52753	402	0
.04636	.03966	.51928	374	0
.05654	.04841	.51417	355	0
.07913	.06770	.50563	326	0
Propionylglycine, m_1/m_2 = 1.000				
0.01003	0.01254	0.55628	498	−35
.02014	.02020	.55357	455	−35
.03022	.03026	.53305	418	−22
.04058	.04067	.52539	392	−29
.07155	.07137	.51095	341	−20
.1034	.1034	.50145	309	−15
m_1/m_2 = 2.000				
0.02006	0.01004	0.54472	461	−35
.04003	.02018	.52619	396	−35
.05980	.03017	.51557	360	−32
.07986	.03995	.50827	335	−31
.1398	.06990	.49384	286	−29
.2002	.1003	.48461	255	−45
Acetyl-DL-alanine, m_1/m_2 = 1.000				
0.01005	0.01007	0.56177	588	−52
.02050	.02062	.54294	520	−42
.03124	.03124	.53206	486	−60
.04031	.04031	.52553	460	−50
.07473	.07474	.50972	410	−40
.1011	.1217	.49719	365	−35
m_1/m_2 = 2.000				
0.02066	0.01045	0.54344	523	−56
.04001	.02000	.52617	464	−48
.06051	.03025	.51532	429	−50
.08147	.04088	.50743	402	−42
.1201	.06030	.49740	368	−45
.1994	.09968	.48452	325	−45
Acetyl-DL-α-amino-*n*-butyric acid, m_1/m_2 = 1.000				
0.01048	0.01086	0.55996	600	−65
.02076	.02152	.54832	538	−36
.03049	.03924	.52652	485	−25
.03584	.03711	.52795	491	−32
.04956	.04969	.52054	467	−38
m_1/m_2 = 2.000				
0.02076	0.01039	0.54378	541	−45
.04053	.02027	.52613	485	−62
.06019	.03009	.51580	449	−45
.07996	.03998	.50846	426	−42
Acetyl-β-alanine, m_1/m_2 = 1.000				
0.01004	0.01004	0.60423	599	0
.02003	.02017	.58647	538	0
.02497	.02498	.58093	522	+20
.03001	.03001	.57635	501	0
.03506	.03508	.57243	487	0
.05015	.05018	.56351	455	0
m_1/m_2 = 2.000				
0.02013	0.01006	0.58646	542	−10
.04027	.02015	.56869	480	−15
.05054	.02529	.56295	458	−10
.06023	.03049	.55827	443	−15
.07044	.03544	.55448	429	−15
.08032	.04022	.55139	419	−15

Calculations and Results

The calculation of the thermodynamic ionization constants of these acids from the electromotive force data was done by the well-known method described by Harned and Owen[6,9] and will not be described here. The results at the two different buffer ratios were extrapolated independently to zero ionic strength. The average values of the negative logarithms of the ionization constants obtained in this way are given in Table III. At the bottom of each column in this table is given the standard deviation of the set of pK values, a measure of the precision of the extrapolations.

TABLE III

THE NEGATIVE LOGARITHMS OF THE IONIZATION CONSTANTS OF SOME N-ACYL AMINO ACIDS

Temp., °C.	Acetyl-glycine	Propionyl-glycine	Acetyl-DL-alanine	Acetyl-DL-α-amino-*n*-butyric acid	Acetyl-β-alanine
5	3.6816	3.7284	3.6992	3.6924	4.4788
10	3.6759	3.7225	3.6992	3.6943	4.4652
15	3.6726	3.7184	3.7026	3.6996	4.4564
20	3.6671	3.7164	3.7075	3.7059	4.4488
25	3.6698	3.7176	3.7152	3.7158	4.4452
30	3.6731	3.7208	3.7248	3.7262	4.4441
35	3.6779	3.7253	3.7334	3.7375	4.4434
40	3.6845	3.7310	3.7450	3.7502	4.4452
45	3.6945	3.7402	3.7589	3.7667	4.4508
50	3.7063	3.7502	3.7735	3.7822	4.4572
Stand. dev.	0.00073	0.00065	0.00067	0.00083	0.00075

The variation of the ionization constants with absolute temperature can be expressed by equations of the form[18]

$$pK = (A/T) + B + CT \qquad (2)$$

All of these constants go through a maximum at an absolute temperature, θ. Values of this quantity and the corresponding minimum values of pK_θ as well as the changes in free energy (ΔF^0), enthalpy (ΔH^0), entropy (ΔS^0) and heat capacity (ΔC_p^0) associated with the ionization reactions in the standard state can be derived from the parameters of equation 2 by conventional thermodynamic methods. The constants A, B and C and these various thermodynamic properties are given in Table IV.

The only one of these acids whose thermodyuamic ionization constant has been reported previ-

(18) H. S. Harned and R. A. Robinson, *Trans. Faraday Soc.*, **36**, 973 (1940).

TABLE IV

PARAMETERS OF EQUATION 2 AND THERMODYNAMIC PROPERTIES ASSOCIATED WITH THE IONIZATION OF N-ACYL AMINO ACIDS

Acid	A	$-B$	C	ΔF^0_{298}, cal. mole^{-1}	ΔH^0_{298}, cal. mole^{-1}	ΔS^0_{298}, cal. deg.$^{-1}$ mole^{-1}	$\Delta C_p{}^0_{298}$, cal. deg.$^{-1}$ mole^{-1}	θ, °K.	pK_θ
Acetylglycine	1248.54	4.8146	0.014411	5006.5	−149	−17.29	−39	294.3	3.6690
Propionylglycine	1101.03	3.7708	.012730	5071.8	−140	−17.49	−35	294.0	3.7168
Acetyl-DL-alanine	908.48	2.8416	.011771	5068.3	−631	−19.12	−32	277.8	3.6986
Acetyl-DL-α-amino-*n*-butyric acid	906.43	2.9315	.012096	5069.3	−773	−19.59	−33	273.7	3.6909
Acetyl-β-alanine	1279.32	3.9494	.013763	6064.1	+255	−19.48	−38	304.9	4.4430

ously is acetylglycine.[19] Neuberger found a pK value of 3.684 at 25° as compared with 3.670 reported here. His calculations involve a double extrapolation and it is believed that our result is more trustworthy.

The reliability of our results may also be judged by consideration of the sources of error. If random errors of ±0.05 mv. in the electromotive forces and standard potentials are assumed and reasonable estimates of the random weighing errors involved in making up the solutions are made, the following *minimum* probable errors are found: ±0.0016 in pK, between ±0.0010 and ±0.0040 in pK_θ, ±2.1 in ΔF^0_{298}, ±16 in ΔH^0_{298}, ±0.06 in ΔS^0_{298}, and ±2 in $\Delta C_p{}^0_{298}$.[9,20] Systematic errors due to thermal electromotive forces and depth of introduction of hydrogen to the cells will cancel because of the use of E^0_w. The error due to change in calibration of the standard cells will also largely cancel since the electromotive forces of cells I and II do not differ greatly. If the standard cells are off by as much as 0.3 mv., which seems unlikely, the maximum error in pK would be 0.0005. The effect of impurities in the acids must also be considered. If the impurity is inert, either non-acidic or with a very low ionization constant, the error in pK is approximately $f(1 + r)/r$ where f is the fraction of undetected impurity and r is m_1/m_2, the buffer ratio. Thus if 0.1% impurity escapes detection, the error in pK is +0.0020 at $r = 1$ and +0.0015 at $r = 2$. We have made measurements at two different buffer ratios for each acid and in all cases the two extrapolated values of pK agree within the expected limits. We have tested the effect of impurity in another way. Three buffer solutions were prepared from crude acetyl-DL-α-amino-*n*-butyric acid (98.9% pure) and measured with the rest. Two of these gave results in good agreement with those based on the repurified material after allowance for the difference in purity; the third deviated by such a large, constant amount as to suggest an accidental error in the preparation of the solution. It is believed that the effect of undetected inert impurities is less than 0.002 in the pK values reported here. None of the systematic errors which have been considered so far would be appreciably temperature-dependent; none should appreciably affect ΔH^0 or ΔC^0_p. The presence of other acids might cause such an error, This appears to be a serious possibility only in the case of acetyl-β-alanine. But our experiments prove the presence of no free amino acids and indicate that other acids are probably absent also.

(19) A. Neuberger, *Proc. Roy. Soc.* (*London*), **A158**, 68 (1937).

(20) N. W. Please, *Biochem. J.*, **56**, 196 (1954). In Please's notation the variance in pK_θ is given by

$$V(pK_\theta) = [(1/m)\rho^2 + (\overline{T}^2/h^2S_1)(\rho - 1)^2 + (\rho^2/S_2h^4)(\theta - \overline{T})^4]\,V(y_0)$$

where $\rho = (\overline{T}/\theta)$ and a term (S_1/m) has been neglected.

Discussion

At any specified temperature the thermodynamic properties are connected by the relation

$$2.3026RT(pK) = \Delta F^0 = \Delta H^0 - T\Delta S^0 \quad (3)$$

For a series of compounds like the fatty acids, the α-amino acids or the N-acyl-α-amino acids pK and ΔF^0 show only minor variations from acid to acid. Considerable variations within a series do occur in both ΔH^0 and ΔS^0, but they are coupled, large negative values of ΔH^0 being associated with large negative values of $T\Delta S^0$ so that pK and ΔF^0 are almost constant.

To assist in placing the data reported in this paper in the proper frame of reference, the free energies, entropies and enthalpies of ionization of various carboxylic acids are shown in Figs. 1–3 as functions of n, the chain length. One purpose of these graphs is to show the effects of chain branching and lengthening in a series of similar compounds like the fatty acids or α-amino acids. For this purpose n is taken as the number of atoms, exclusive of hydrogens and the carboxyl group, in the longest chain of the acid molecule. Many of the acid molecules also contain polar substituents. In these cases groups such as hydroxyl, chlorine, N-acyl, N-glycyl and carboxyl have been counted as contributing one unit to n when they come at the end of a chain. On this basis, for example, the chain length is taken to be two units for propionic acid, glycine, DL-alanine, DL-serine, glycylglycine, chloroacetic acid, the hydrogen malonate ion and all of the N-acyl α-amino acids.[21]

The values of the thermodynamic properties presented in Figs. 1–3 are those at 25°. This reference temperature has been chosen because it lies in the middle of the experimental temperature range where the properties are known with the greatest precision. Examination of the corresponding data at 10 and 50° reveals only minor variation from the relations shown at 25°. Nevertheless it is always well to keep in mind when examining small fluctuations in the thermodynamic properties that the choice of 25° is an arbitrary one. To illustrate, the ionization constants and free energies of ionization of acetyl-DL-alanine, propionylglycine and acetyl-DL-α-amino-*n*-butyric acid are

(21) Similar graphs have been given by Everett[9,22] except that n was taken as the total number of carbon atoms in the molecule exclusive of the one in the carboxyl group.

(22) D. H. Everett, *Ind. chim. belge*, **16**, 647 (1951).

virtually identical at 25° but the agreement is less close at other temperatures.

An attempt has been made to indicate in Figs. 2 and 3 the relative reliability of the enthalpy and entropy changes. These estimates, often only rough guesses, have been based in many cases on an examination and sometimes a recalculation of the original data. Most of the data are from measurements on cells without liquid junction,[23] some are from measurements which require an extrapolation to eliminate the liquid junction potential,[24] and a few directly measured heats of ionization are included.[25] Due attention was paid in estimating the reliability of these data to the inherent precision of the extrapolations and to such details as purity of compounds and exclusion of oxygen. Limitations of space preclude a detailed discussion here. It should be noted carefully that the size of the circles in Figs. 2 and 3 represents more closely an estimate of precision rather than one of accuracy. Probable errors of two or three times the indicated precisions are to be expected in some cases. Nevertheless, in a series of acids all studied in the same way, it may still be possible to make valid comparisons of one acid with another, for systematic errors will tend to cancel.

The thermodynamic property with which we shall be least concerned in the following discussion is the change in heat capacity. The probable error in ΔC^0_p is at least ± 2 cal. deg.$^{-1}$ mole.$^{-1}$ and variations in this property from acid to acid are not sufficiently pronounced to permit reliable deductions.

The Free Energy and Entropy of Ionization.—In principle, the logical starting point in a search for relations between structure and properties should be provided by statistical mechanics. Let ΔE^0_0 represent the sum total of contributions to the free energy of ionization resulting from the zero point vibrational energies and such potential energy terms as are independent of temperature. The latter may include the binding energy of the proton to oxygen in the acidic hydroxyl group, resonance energy, induction energy and the like.[26] The grosser differences in acid behavior have often been interpreted in terms of inductive, polar and resonance effects which are related to these potential energy contributions to ΔE^0_0.[27] The experimental temperature range is much too far removed from absolute zero to permit the determination of ΔE^0_0 by extrapolation.[28] Consequently, it is nec-

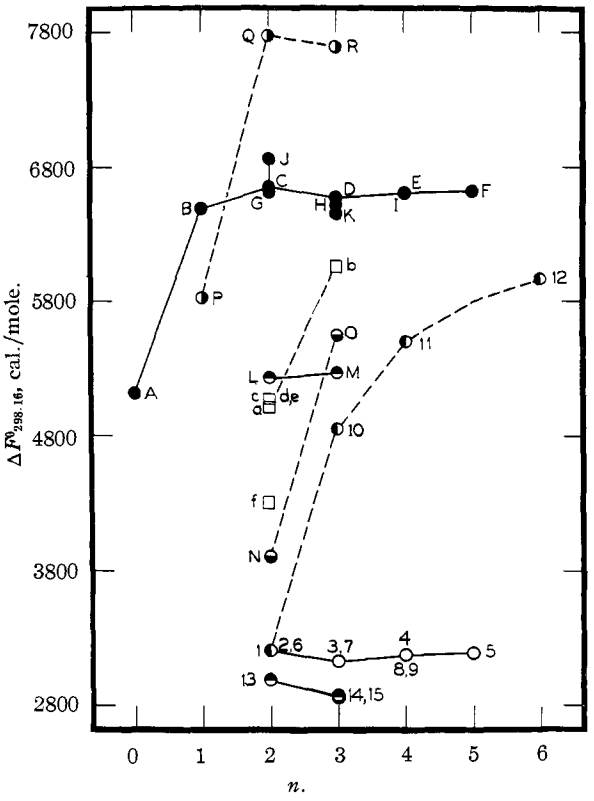

Fig. 1.—The free energies of ionization of carboxylic acids at 25° as a function of chain length. α-Amino acid hydrochlorides, ○: 1, glycine; 2, DL-alanine; 3, DL-α-amino-*n*-butyric acid; 4, DL-norvaline; 5, DL-norleucine; 6, α-aminoisobutyric acid; 7, DL-valine; 8, DL-leucine; 9, DL-isoleucine. ω-Amino acid hydrochlorides, ◐: (1, glycine); 10, β-alanine; 11, γ-aminobutyric acid; 12, ε-aminocaproic acid. Fatty acids, ●: A, formic acid; B, acetic acid; C, propionic; D, *n*-butyric; E, *n*-valeric; F, *n*-hexoic; G, isobutyric; H, isovaleric; I, isohexoic; J, trimethylacetic; K, diethylacetic acid. Hydroxy-substituted acids, ◓: 13, DL-serine; 14, DL-threonine; 15, DL-allothreonine; L, glycolic acid; M, lactic acid. Chloro-substituted acids, ◒: N, chloroacetic acid; O, β-chloropropionic acid. N-Acyl amino acids and peptides, □: a, acetylglycine; b, acetyl-β-alanine; c, propionylglycine; d, acetyl-DL-alanine; e, acetyl-DL-α-amino-*n*-butyric acid; f, glycylglycine. Anion acids, ◑: P, hydrogen oxalate ion; Q, hydrogen malonate ion; R, hydrogen succinate ion. Solid lines connect acids in which the polar groups, if present, are at fixed locations. Broken lines connect acids in which the position of the polar group varies.

(23) Glycine: B. B. Owen, THIS JOURNAL, **56**, 24 (1934) and ref. 7, DL-alanine: L. F. Nims and P. K. Smith, *J. Biol. Chem.*, **101**, 401 (1933) and P. K. Smith, A. C. Taylor and E. R. B. Smith, *ibid.*, **122**, 109 (1937); DL-α-amino-*n*-butyric acid, DL-norleucine, DL-norvaline, α-aminoisobutyric acid, DL-valine, DL-leucine and DL-isoleucine: P. K. Smith, A. C. Taylor and E. R. B. Smith, above; β-alanine: M. May and W. A. Felsing, THIS JOURNAL, **73**, 406 (1951); γ-aminobutyric acid: ref. 9; ε-aminocaproic acid and glycylglycine: E. R. B. Smith and P. K. Smith, *J. Biol. Chem.*, **146**, 187 (1942); DL-serine, DL-threonine, and DL-allothreonine: P. K. Smith, A. T. Gorham and E. R. B. Smith, *ibid.*, **144**, 737 (1942); formic acid: H. S. Harned and N. D. Embree, THIS JOURNAL, **56**, 1042 (1934); acetic acid: H. S. Harned and R. W. Ehlers, *ibid.*, **55**, 652 (1933); propionic acid: H. S. Harned and R. W. Ehlers, *ibid.*, **55**, 2379 (1933); *n*-butyric acid: H. S. Harned and R. O. Sutherland, *ibid.*, **56**, 2039 (1934); glycolic acid: L. F. Nims, *ibid.*, **58**, 987 (1936); lactic acid: L. F. Nims and P. K. Smith, *J. Biol. Chem.*, **113**, 145 (1936); chloroacetic acid: D. D. Wright, THIS JOURNAL, **56**, 314 (1934); hydrogen oxalate ion: H. S. Harned and L. D. Fallon, *ibid.*, **61**, 3111 (1939) and G. D. Pinching and R. G. Bates, *J. Research Natl. Bur. Standards*, **40**, 405 (1948); hydrogen malonate ion: W. J. Hamer, J. O. Burton and S. F. Acree, *ibid.*, **24**, 269 (1940); hydrogen succinate ion: G. D. Pinching and R. G. Bates, *ibid.*, **45**, 322 (1950).

(24) Propionic acid, *n*-valeric acid, *n*-hexoic acid, isobutyric acid, isovaleric acid, isohexoic acid, trimethylacetic acid and diethylacetic acid: ref. 3; β-chloropropionic acid: E. Larsson, *Z. physik. Chem.*, **A165**, 53 (1933).

(25) Glycine: J. Sturtevant, THIS JOURNAL, **63**, 88 (1941); DL-alanine: J. Sturtevant, *ibid.*, **64**, 762 (1942); β-chloropropionic acid: T. L. Cottrell, G. W. Drake, D. L. Levi, K. J. Tully and J. H. Wolfenden, *J. Chem. Soc. (London)*, 1016 (1948).

(26) G. Briegleb, *Z. Naturforsch.*, **4a**, 171 (1949).

(27) L. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1940, p. 77.

(28) D. H. Everett and W. F. K. Wynne-Jones, *Trans. Faraday Soc.*, **35**, 1380 (1939); G. Briegleb, *Naturwiss.*, **31**, 62 (1943).

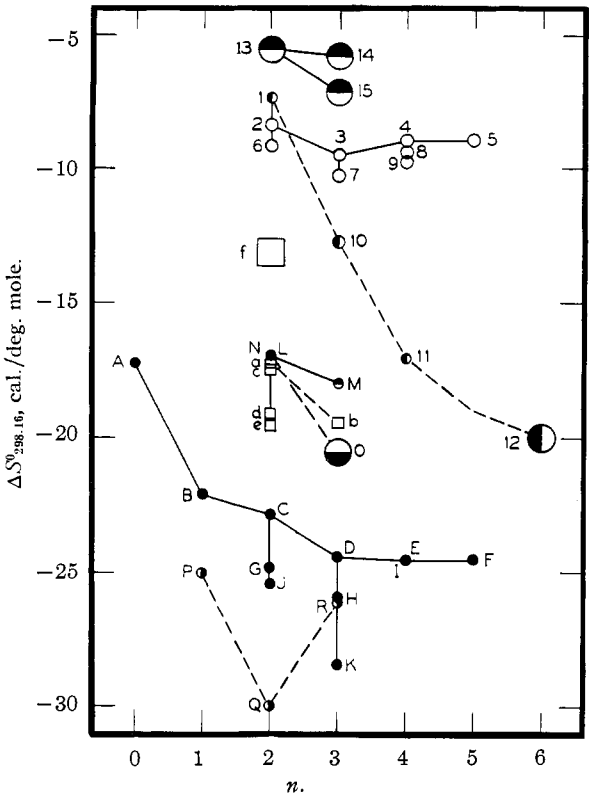

Fig. 2.—The entropies of ionization of carboxylic acids at 25° as a function of chain length.

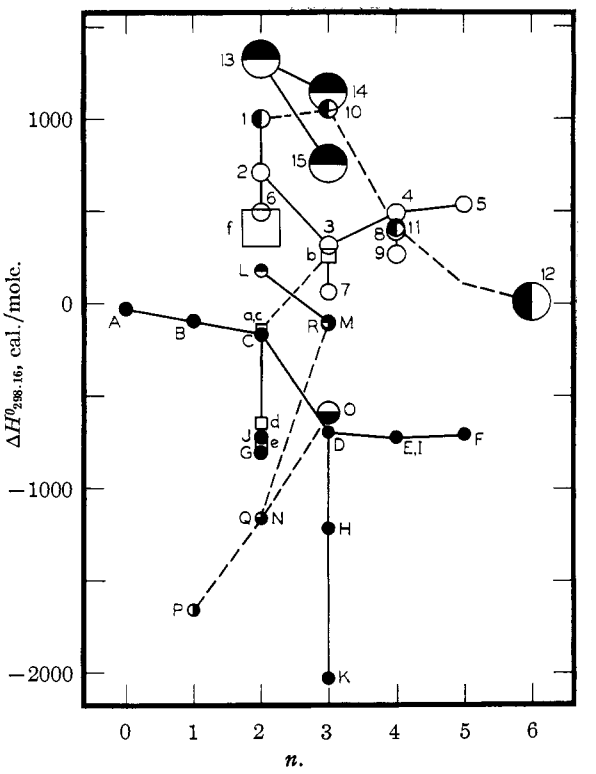

Fig. 3.—The enthalpies of ionization of carboxylic acids at 25° as a function of chain length.

essary to fall back on the conclusion of Evans and Polanyi[29] that variations in ΔE^0_0 from one reaction to another are more closely matched by variations in ΔF^0_T than by those in ΔH^0_T. From this point of view the acyl α-amino acids, all of which have about the same value of the free energy of ionization at 25°, should have the same value of ΔE^0_0. This is reasonable since the location of the polar peptide linkage with respect to the carboxyl group is the same in all four of these acids. By contrast, acetyl-β-alanine is a weaker acid with values of ΔF^0_{298} and ΔE^0_0 nearer those of the unsubstituted fatty acids because the peptide linkage is further removed from the ionizing group.

That the free energy, and not the heat of ionization, is more closely related to ΔE^0_0 can be illustrated by the observation that the heats of ionization of the fatty acids and comparable acyl amino acids are almost identical. The higher heat of ionization of acetyl-β-alanine as compared with that of acetylglycine (a and b in Fig. 3) is to be expected because of the removal of the peptide linkage further from the carboxyl group. Comparable increases in heat of ionization are found between chloroacetic and β-chloropropionic acids (N and O) and between hydrogen malonate and hydrogen succinate ions (Q and R).

Up to this point the effect of molecular motions as expressed in partition functions has not been considered. The entropy of ionization is of particular interest in this connection since it does not depend on ΔE^0_0. A successful treatment of the entropies of some neutral molecules, monatomic ions, and roughly spherical oxyanions has been based on partition functions for a smoothed-potential model of liquids,[30,31] but the applicability of this model to the complex acids under consideration is open to question. In any case, owing to cancellation of contributions from the un-ionized and ionized forms of the acid in computing the entropy of ionization, the conventional contributions due to translation, rotation, vibration and free volume probably do not produce fluctuations of ΔS^0 for a series of similar acids exceeding 0.2 cal. mole^{-1} deg.$^{-1}$. Almost all of the entropy of ionization must arise from interactions between solute and solvent which lead to special orientations of solvent molecules about solute particles and also to restrictions on the internal rotations of these particles. That an adequate treatment of the free energy of ionization is sometimes possible without a detailed description of solute–solvent interaction is illustrated by the success of the electrostatic theory of Kirkwood and Westheimer.[32] Estimates of the entropy of ionization based on this theory are far too low, and Kirkwood and Westheimer have suggested that this is due to their neglect of the effect of the ions on the structure of water.[33] Though the molecular shapes of the acyl amino acids are not favorable ones for a quantitative treatment using their model, the

(29) M. G. Evans and M. Polanyi, *Trans. Faraday Soc.*, **32**, 1333 (1936).

(30) R. E. Powell and W. M. Latimer, *J. Chem. Phys.*, **19**, 1139 (1951).

(31) R. E. Powell, *J. Phys. Chem.*, **58**, 528 (1954).

(32) J. G. Kirkwood and F. H. Westheimer, *J. Chem. Phys.*, **6**, 506, 513 (1938); F. H. Westheimer and M. H. Shookhoff, THIS JOURNAL, **61**, 555 (1939).

(33) F. H. Westheimer and J. G. Kirkwood, *Trans. Faraday Soc.*, **43**, 77 (1947).

method of Kirkwood and Westheimer could provide, in principle, a reasonable point of view for an explanation of the lower free energies of ionization of the acyl amino acids as compared with the fatty acids. In considering entropies of ionization we are, of necessity, reduced to more qualitative considerations.

Water Orientation Effects.—The orientation of water about simple, spherical ions gives rise to a compressed sheath of water with lower entropy.[34-38] Outside of this primary hydration sheath a disordered or depolymerized region of abnormally high entropy probably occurs until at larger distances from the ion the normal loose-packed, semicrystalline structure of water is re-established.[39] The solvation of simple ions is accompanied by a rough coupling of heat and entropy effects, *i.e.*, large negative heats of solvation are associated with large negative entropies of solvation.[39]

Much less is known about the arrangement of water molecules about a planar, hydrogen-bonding group like the carboxylate ion, but it certainly produces a strong ordering of neighboring water molecules.[40,41] The effective range of influence of the carboxylate and ammonium ions on the structure of water appears to be about 5 Å.[3,42] This would place the peptide linkage in the acyl α-amino acids well within the sphere of influence of the carboxylate ion while the peptide linkage of acetyl-β-alanine would be near the outer limit of this influence. Some orientation of water molecules about uncharged carboxyl, hydroxyl and amino groups is to be expected because of hydrogen bonding, but this is apparently much weaker than that about charged groups.[41]

Many of the more marked differences in entropy of ionization have been explained on the basis of preferential solvent orientation about charges with consequent loss of entropy.[3,42] The ionization of fatty acids, for example, is ionogenic and produces a large negative entropy of ionization because of the strong orientation of water about the ions. In the ionization of α-amino acid hydrochlorides the charged ammonium group binds water both to the un-ionized and to the ionized forms so that less water can be influenced by the carboxylate ion and the entropy of ionization is considerably less negative than that of a fatty acid. The peptide linkage in the larger solid glycyl peptides has been found to bind water,[2] but adsorption of water by the smaller crystalline peptides is probably prevented by saturation of the hydrogen-bonding power of the groups in the crystal. There is no reason to expect such saturation to occur in solution. Orientation of water about the peptide linkage should be aided by slight charges, roughly four-tenths of a unit charge,[43] borne by the oxygen and nitrogen. Water bound to the peptide linkage is within 5 Å. of the carboxylate group and it is therefore reasonable to expect the entropy of ionization of acetylglycine or propionylglycine to be less negative than that of propionic acid but a good deal more negative than that of glycine, as is indeed the case.

Examination of Fig. 2 reveals that a number of acids have entropies of ionization which are 5 cal. deg.$^{-1}$ mole^{-1} less negative than those of the fatty acids: chloroacetic acid ($\Delta S^0_{298} = -16.97$), glycolic acid (−16.94), acetylglycine (−17.29), propionylglycine (−17.49) and formic acid (−17.24). Chloroacetic and glycolic acids, like the two acyl amino acids, have strongly polar groups attached to the α-carbon atom. The direct electrostatic effect of these on the carboxyl group is largely exerted within the molecule itself and not through the solvent.[32] Contributions to the entropy from this source are therefore probably small and solvent orientation about the polar groups must be largely responsible for the small entropies of ionization of these acids as compared with the fatty acids. Some difference in entropies of ionization is to be expected also because of restricted rotation. In the fatty acids the internal rotations of the alkyl chain are restricted after ionization.[3] In the polar acids under consideration considerable restriction of rotation may exist already in the un-ionized form owing to a combination of steric and electrostatic effects, with the result that less entropy is lost on ionization. Such effects, which probably amount to no more than one entropy unit, will be discussed in the next section. A further example of a change in entropy of ionization of about 5 units by introduction of polar groups is noted in comparing succinic acid ($\Delta S^0_{298} = -16.68$ cal. deg.$^{-1}$ mole^{-1})[44] with *d*-tartaric acid (−11.40).[45] On the other hand, the five unit differences in entropies of ionization of glycine and β-alanine (1 and 10 in Fig. 2) and of hydrogen oxalate and hydrogen malonate ions (P and Q) are clearly reflections of differences in charge interaction and solvent orientation effects arising from increasing the distance between charged groups.

Formic acid occupies a special position. It is instructive to compare the entropy changes in the reactions

$$CH_3COOH + HCOO^- \longrightarrow HCOOH + CH_3COO^-; \quad \Delta S^0_{298} = -4.9 \text{ cal. deg.}^{-1} \text{ mole}^{-1} \quad (A)$$

$$CH_3NH_3^+ + NH_3 \longrightarrow NH_4^+ + CH_3NH_2; \quad \Delta S^0_{298} = -3.7 \text{ cal. deg.}^{-1} \text{ mole}^{-1} \quad (B)$$

The two entropy changes are of the same order of magnitude and might, indeed, be still closer together if contributions from moments of inertia and restricted internal rotations of the methyl groups in the acetate and methylammonium ions were taken into account.[46] It is difficult to see how these

(34) J. D. Bernal and R. H. Fowler, *J. Chem. Phys.*, **1**, 515 (1933).
(35) D. D. Eley and M. G. Evans, *Trans. Faraday Soc.*, **34**, 1093 (1938).
(36) D. H. Everett and C. A. Coulson, *ibid.*, **36**, 633 (1940).
(37) E. J. Verwey, *Rec. trav. chim.*, **61**, 127 (1942).
(38) D. D. Eley, *Trans. Faraday Soc.*, **40**, 184 (1944).
(39) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).
(40) R. G. Gurney, "Ionic Processes in Solution," McGraw-Hill Book Co., New York, N. Y., 1953, pp. 169, 176.
(41) G. H. Haggis, J. B. Hasted and T. J. Buchanan, *J. Chem. Phys.*, **20**, 1452 (1952).
(42) D. H. Everett and B. R. W. Pinsent, *Proc. Roy. Soc.* (*London*), **215A**, 416 (1952).
(43) G. B. Carpenter and J. Donohue, THIS JOURNAL, **72**, 2315 (1950).
(44) G. Pinching and R. G. Bates, *J. Research Natl. Bur. Standards*, **45**, 444 (1950).
(45) R. G. Bates and R. G. Canham, *ibid.*, **47**, 343 (1951).
(46) The entropy change of −3.7 units in reaction B is the observed entropy change (R. G. Bates and G. D. Pinching, *ibid.*, **42**, 419 (1949); D. H. Everett and W. F. K. Wynne-Jones, *Proc. Roy. Soc.* (*London*), **177A**, 499 (1941)) increased by 0.57 unit to correct for the different symmetry numbers.

entropy changes can have a common cause and particularly a cause originating in the same kind of ion–solvent interactions, for the comparable ions come on opposite sides of the two equations. The entropy change in the second reaction has been attributed to a solvent exclusion effect which makes the entropy of the methylammonium ion less negative than that of the ammonium ion.[4,47] Alternatively, this can be expressed as a decrease in hydrogen-bonding power of the ammonium ion by introduction of the methyl group.[48] But solvent exclusion would tend to make the entropy change of reaction A positive. Entropy effects associated with restriction of rotation of the methyl groups in the acetate and methylammonium ions would also be of the opposite sign. An explanation of the similar entropy changes of reactions A and B must be based upon a more definite picture of the structure of water about alkyl groups and the carboxylate and ammonium ions.

That alkyl groups have an effect on the structure of water is shown by the large decrease in entropy and increase in heat capacity associated with the introduction of the smaller saturated hydrocarbons in water.[39] The orientation of water about alkyl groups, which gives rise to a restricted librational motion of the water molecules[30] and loss of entropy, may resemble the arrangement of water in the solid hydrates of the hydrocarbons.[49] These structures, which are consistent with the X-ray diffraction data,[50] are open patterns of water molecules with only slight distortion of the hydrogen bonds from the tetrahedral angle.

The roughly spherical ammonium ion is surrounded by a primary hydration shell containing about four tightly held water molecules.[41] The replacement of one hydrogen of the ammonium ion by a methyl group displaces a water molecule from this first hydration shell, increases the entropy of the hydrated ion and makes the entropy of ionization of the methylammonium ion more negative than that of the ammonium ion itself. If the methyl group is replaced by an ethyl group, the entropy of ionization is found to be *less* negative. This can occur if the outer end of the ethyl group penetrates the envelope of disordered water molecules[39] outside the primary hydration shell of the ion. The alkyl group through its displacement of high-entropy water and its structure-strengthening ability will decrease the entropy of the ion. For the same reason the entropies of ionization of the di- and triethylammonium ions are less negative than those of the corresponding methyl derivatives.

The planar, triangular-shaped carboxylate group will certainly not give rise to the same orientation of solvent as the ammonium ion does. The carboxylate group probably binds about six water molecules in the primary hydration shell.[41] These water molecules can be arranged so that the "back" of the anion is outside of the tightly bound primary layer. Displacement of solvent from this layer would then not occur as a result of introduction of a methyl group. It is reasonable, on the other hand, to suppose that the outlying disordered region of solvent would extend around the hydrogen in back of the formate ion. The methyl group in the acetate ion would then protrude into the disordered region rather than into the primary hydration shell. This will make the entropy of the acetate ion more negative than that of the formate ion because the methyl group displaces water from the disordered region and reduces its positive contribution to the entropy and the methyl group by virtue of its structure-strengthening ability causes an ordering of water molecules in the disordered region with a decrease in their entropy. Hence, the entropy of ionization of acetic acid will be more negative than that of formic acid. The formate ion from this point of view is less order-producing than the acetate ion and this is consistent with its higher mobility.[51] With the propionate ion the ethyl group will penetrate further into the disordered region and give rise to a still more negative entropy of ionization. If the range of influence of the carboxylate group is about 5 Å.[3] and if the degree of disorder is greatest near the primary hydration layer, it is logical to expect the increase in size of the alkyl group from methyl to ethyl to cause a considerably smaller change in entropy of ionization than the introduction of the methyl group in the first place and this is the case.

It is thus possible to offer a plausible explanation of the similar entropy changes of reactions A and B, though a different type of ion–solvent interaction must be involved in each case. A similar interpretation of the changes in heat capacity associated with these two reactions is also possible, but the experimental uncertainty in these quantities is so large that a detailed discussion is hardly worthwhile. Another feature of this picture is less satisfying. The small change in heat of ionization between formic and acetic acids does not seem consistent with the close coupling of heat and entropy changes for the solvation of hydrocarbons or other nonpolar molecules like the noble gases.[39] In this respect the transition from formic acid to acetic acid is different from the transitions from straight chain to branched chain or short chain to long chain acids discussed in the next section. That this necessarily indicates a different origin of the entropy changes for the latter transitions can hardly be proved or disproved until specification of solute–water interactions can be made more precise and quantitative. In the treatment of chain length and chain branching effects in the next section we shall consider not only water–alkyl group interactions of the type described above but also the possible effect of restricted rotation of the alkyl groups.[3,4,22]

Chain Branching and Lengthening Effects.—A number of fairly small entropy effects are associated with branching and lengthening of alkyl chains in homologous acids. The replacement of a hydrogen atom on the α-carbon atom by a methyl group invariably results in a decrease of from 1 to 2 cal. mole^{-1} deg.$^{-1}$ in the entropy of ionization. This is

(47) G. Briegleb, *Z. Elektrochem.*, **53**, 350 (1949).

(48) A. F. Trotman-Dickenson, *J. Chem. Soc.* (*London*), 1296 (1949).

(49) W. F. Claussen, *J. Chem. Phys.*, **19**, 662, 1425 (1951); W. F. Claussen and M. F. Polglase, THIS JOURNAL, **74**, 4817 (1952).

(50) M. von Stackelberg and H. R. Müller, *J. Chem. Phys.*, **19**, 1319 (1951).

(51) Ref. 40, p. 201.

illustrated by the transitions from propionic acid to isobutyric acid (C to G in Fig. 2), glycolic acid to lactic acid (L to M), glycine to α-alanine (1 to 2) and acetylglycine to acetyl-DL-alanine (a to d). Substitution of a second methyl group for hydrogen on the α-carbon atom causes a further but smaller decrease, *e.g.*, isobutyric acid to trimethylacetic acid (G to J) and α-alanine to α-aminoisobutyric acid (2 to 6). Chain branching farther away from the carboxyl group is associated with still smaller changes in entropy. Lengthening of the alkyl chain also is accompanied in some cases by entropy decreases. The effect is most noticeable when the chain length is increased from two to three carbon atoms, *e.g.*, propionic acid to butyric acid (C to D), isobutyric acid to isovaleric acid (G to H), alanine to α-amino-*n*-butyric acid (2 to 3), α-aminoisobutyric acid to valine (6 to 7) and serine to threonine or allothreonine (13 to 14 or 15).

These effects are closely associated with the presence of charged groups. When chain branching or lengthening occurs beyond 5 Å. of the carboxylate group, little or no change in the entropy of ionization is found, *e.g.*, isohexoic *vs.* *n*-valeric acid (I and E). The ionization of the ammonium group in amino acids, a reaction which involves the loss of ionic charge, is associated with positive chain-branching effects in contrast to the negative ones described above for reactions in which charge fields are created.

For both the chain-branching and chain-lengthening effects there is a coupling of heat and entropy changes. If, for example, branching at the α-carbon atom causes a more negative value of $T\Delta S^0$, this is associated with a more negative value of ΔH^0, so that ΔF^0 is affected only slightly. The coupling is closest for a given chain length, n, and is particularly close in the α-amino acid series where the presence of the charged ammonium group in both the un-ionized and ionized acid probably swamps minor variations. The coupling is least evident for the increase in chain length from $n = 2$ to 3; this is almost invariably associated with a small decrease in ΔF^0. These couplings of heat and entropy changes are reminiscent of the linear relation between heats and entropies of solvation of gaseous atoms, molecules and ions.[39,52,53]

The origin of these effects can be sought in solvent orientation and restricted rotation. It has been suggested that the solvent exclusion effect is responsible for the entropy changes due to chain branching,[22] but this cannot be an important factor since it would tend to make the changes less negative whereas more negative values of ΔS^0 are observed. The order-producing interaction of alkyl groups with water discussed in the preceding section would also produce less negative entropies of ionization by chain branching if the alkyl groups were within the innermost hydration shell about the carboxylate ion. If it be supposed, as was done in the discussion of formic and acetic acids, that this shell does not extend in back of the ionic group, then the alkyl groups may lie in the disordered or depolymerized region outside this innermost shell. This is certainly possible if the alkyl group branches from the α-carbon atom. Even if the primary hydration shell does extend back to some extent so that the disordered region starts as far back as 2.8 Å., the oxygen–oxygen distance in ice,[39] a methyl group on the α-carbon atom could still partially project into the disordered region. Now the disordered region is created in the ionization process, and the alkyl group because of its structure-strengthening ability will counteract this disorder making the positive contribution of the disordered region to the entropy of the ion smaller. The entropy of ionization of a branched chain acid should thus be more negative than that of an unbranched acid. Replacement of an α-methyl group by ethyl should cause greater penetration of the disordered region and a further decrease in the entropy of ionization. This change will be smaller than that attending the original introduction of the methyl group because the degree of disorder is probably greatest nearest the primary hydration shell. Thus the entropy of ionization of acetyl-DL-α-amino-*n*-butyric acid is only 0.47 cal. mole^{-1} deg.$^{-1}$ more negative than that of acetyl-DL-alanine whereas the latter is 1.8 units more negative than that of acetylglycine. The close coupling of heat and entropy changes associated with chain branching is consistent with the coupling of these changes for solvation of the simple hydrocarbons.[39,52,53] Our knowledge of the arrangement of water molecules about a carboxylate ion is not sufficient to enable us to push this description further.

Another possible cause of the anomalous chain branching and lengthening effects can be sought in restrictions on internal rotations that arise from the creation or disappearance of charged groups by the ionization reaction.[3,4,22] With gaseous and liquid phases it has generally been found that steric repulsion is the primary cause of restricted rotation and electrostatic forces have only a secondary effect.[54] Hill[55] has made some calculations of the influence of ionic charges on internal rotations of solutes in aqueous solution based on electrostatic interaction through a structureless continuum. He concluded that the effect was of secondary importance though it might approach in magnitude the non-electrostatic contribution to restricted rotation in certain cases if local variations in the effective dielectric constant at different orientations about the ion were considered. But charged groups may cause restriction of rotation in a different way: the compressed, tightly bound water about charged groups, whether in the primary hydration layer or in the outer disordered zone, could restrict rotation of alkyl groups imbedded in this region.[3,4] In view of our lack of detailed knowledge of the structure of water and the nature of restricted rotation, quantitative calculations can hardly be made. But the hypothesis that restricted rotation is responsible for the chain lengthening and branching effects can be tested by rough estimates and qualitative comparison with the data.

The effect of substitution of methyl for hydrogen

(52) R. P. Bell, *Trans. Faraday Soc.*, **33**, 496 (1937).

(53) I. M. Barclay and J. A. V. Butler, *ibid.*, **34**, 1445 (1938).

(54) S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press, Inc., New York, N. Y., 1954, Chapters III, IV and VI.

(55) T. Hill, *J. Chem. Phys.*, **11**, 545, 552 (1943); **12**, 56, 147 (1944); THIS JOURNAL, **73**, 5304 (1951).

on the α-carbon atom is a test case. The methyl group is well within the 5 Å. limit of influence[3] of the carboxylate group on the water structure. It is thus subject in the anion of the acid to restriction both from direct electrostatic interaction and from being imbedded in a rigid water sheath about the charged group. Consider, as a crude approximation, the methyl group of acetyl-DL-alanine to be a symmetrical top affixed to a rigid frame.[56] Then with reasonable guesses for the restricting potentials (1 to $4RT$ before ionization and 8 to $16RT$ afterwards) it is easy to predict an entropy decrease accompanying ionization due to restricted rotation of 1 to 2 cal. deg.$^{-1}$ mole^{-1}. This is comparable with the 1.8 unit difference between the entropies of ionization of acetylglycine and acetyl-DL-alanine. The contribution of restricted rotaton to ΔC^0_p is small and probably positive, that to ΔH^0 is negative. Both effects are of the correct sign but are smaller than the experimental data require. The coupling of heat and entropy changes is not close, so that the contribution of restricted rotation to ΔF^0 is larger than that observed. The crudities of the model must prevent us from placing too much weight on these calculations, but they do indicate that restricted rotation can give rise to contributions to the thermodynamic properties which are in the right direction and the effect on ΔS^0 is of the right order of magnitude.

Other observations are consistent with the hypothesis. Chain branching on carbon atoms further removed from the carboxylate group leads to smaller effects as the methyl group is withdrawn from the tight water sheath about the charge. Substitution of an ethyl group for methyl on the α-carbon atom results in a still more negative entropy of ionization, because the ethyl group has more rotational degrees of freedom to be restricted. For this reason the entropy of ionization of acetyl-α-amino-*n*-butyric acid could be and is more negative than that of acetylalanine.

The changes in entropy of ionization associated with lengthening of the alkyl chain in substituted ammonium ions and fatty acids have been attributed to restriction of rotation about carbon–carbon bonds in the chain caused by the tight binding of water about the charged group.[3,4] In acids with a chain three carbon atoms long, rotational isomerism[54] about the α- and β-carbon atoms should be possible. If ionization stabilizes one of the isomers, perhaps the *trans* configuration, loss of entropy associated with the mixture of isomers could result. This is a possible explanation for the more negative entropies of ionization of acids with $n = 3$ as compared with acids with shorter chains. The acyl α-amino acids are interesting in this connection because of the stiffness of the peptide linkage.[5,43,54,57] The two carbonyl groups are frozen in the *trans* configuration regardless of the state of ionization of the acid. Since there remain fewer rotational modes to be frozen out by ionization, these acids should have less negative entropies and enthalpies of ionization than do the long chain fatty acids. A possible stabilization by ionization of one of the rotational isomers about the α- and β-carbon atoms in acetyl-β-alanine could make its entropy of ionization more negative than those of acetyl- or propionylglycine. None of these contributions to the entropy of ionization due to restricted rotation should exceed 2 cal. deg.$^{-1}$ mole^{-1} and contributions of 1 unit or less are most likely. They cannot be separated from water-orientation effects which they will tend to reinforce.

The Temperature of Maximum Ionization Constant.—The ionization constants of practically all carboxylic acids reach maximum values at temperatures between 0° and 60°. The absolute temperature, θ, corresponding to this maximum is related to ΔH^0 at a specified temperature T by

$$AH^0_T = 2.3026RC(\theta^2 - T^2) \qquad (4)$$

The constant C is a parameter of equation 2 and is related to ΔC^0_p. Since C does not vary much from acid to acid, it follows that at any specified temperature, *e.g.*, $T = 298.16$°K., variations in ΔH^0 will be matched roughly by corresponding ones in θ.[58] The values of θ, like those of ΔH^0_{298}, are about the same for comparable fatty acids and acyl amino acids. Chain branching at the *alpha*-carbon atom in either series produces a drop of 15 to 20° in θ. This large change is all the more remarkable when it is noted that the difference in θ values of a fatty acid and an *alpha*-amino acid, two acids of different charge type, is only about 30°.

Gurney[59] has developed an expression for θ on the assumption that the work required to remove a proton from the acid molecule is separable into a part independent of temperature (J_{non}) and an electrostatic contribution (J_{el}) which varies with the reciprocal of the dielectric constant of water

$$\theta = k[1 + (J_{non}/J_{el}(\theta))] \qquad (5)$$

where k is a constant characteristic of the pure solvent. However successful this approach may be for a description of the behavior of acids of radically different type, it is manifestly inadequate as the basis for interpreting differences in θ values of similar acids. It does not seem reasonable, for example, to try to account for the large change in θ produced by introduction of a methyl group on the α-carbon atom by either an increase in J_{el} or a decrease in J_{non}. All expressions, including equation 5, which attempt to abbreviate the contributions of solute–solvent interaction to a simple electrostatic effect through a structureless dielectric are inadequate for the treatment of similar acids. A more explicit accounting of water orientations, including their effect on restricted rotation, is required. The importance of solvent orientation may be illustrated by the observation that the difference in temperatures of maximum ionization constant between acetic acid in water and deuteroacetic acid in deuterium oxide[60] is the same, within the experimental error, as the difference in the temperatures

(56) K. S. Pitzer, "Quantum Chemistry," Prentice–Hall, Inc., New York, N. Y., 1953, Appendix 18.

(57) W. D. Phillips, *J. Chem. Phys.*, **23**, 1363 (1955).

(58) A similar prediction can be made when the ionization constants are fitted to the equation[28] $\log K = (A/T) + B \log T + C$; in that case $\Delta H^0_T = RB(T - \theta)$.

(59) Ref. 40, pp. 128–132.

(60) F. Brescia, V. K. LaMer and F. C. Nachod, THIS JOURNAL, **62**, 614 (1940).

of maximum density of the pure solvents. The latter property is an indication of the relative structural strengths of the two liquids.

The suggestion has been made by Harned and Embree[61] that comparisons of acid strength should be made at θ rather than at some arbitrary temperature like 298°. There appears to be no advantage in this so far as pK_θ values are concerned, and comparisons of $\Delta F^0_\theta = -2.3026R\theta(pK_\theta)$ are not at all as favorable as those at some constant temperature because of the variable factor θ. The entropy change at θ shows the same behavior as pK_θ (or even pK_{298}) since $\Delta S^0_\theta = 2.3026R(pK_\theta)$.[60] Thus chain branching and lengthening effects all but disappear when entropy changes are compared at θ°. At 298.16°K. the entropy of ionization of acetyl-DL-alanine is 1.8 units more negative than that of acetylglycine. At 277.4°K. the temperature of maximum ionization constant for the branched chain acid, the ice-like ordered structure of the solvent predominates and less change in orientation of water can be produced as a result of ionization. The entropy of ionization of acetylalanine at this temperature therefore is less negative than that at 298.16° and differs from that of acetylglycine at its temperature of maximum ionization (294.3°) by only 0.13 unit.

NEW YORK, N. Y.

(61) H. S. Harned and N. D. Embree, THIS JOURNAL, **56**, 1050 (1934).

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An Exact Solution of the Equations for Free Diffusion in Three-component Systems with Interacting Flows, and its Use in Evaluation of the Diffusion Coefficients

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RECEIVED SEPTEMBER 26, 1955

A rigorous solution of the differential equations for one-dimensional free diffusion is obtained for a three-component system in which the solute flows interact; the only assumptions are that the volume change on mixing and the concentration dependence of the diffusion coefficients are negligibly small. Based on this solution, a general procedure is developed for computing the four diffusion coefficients from data for the reduced height–area ratios, $\mathfrak{D}_A$, and reduced second moments, $\mathfrak{D}_{2m}$, of the refractive index gradient curves of two or more experiments. An additional procedure for calculating the coefficients is devised which depends primarily on measurements of $\mathfrak{D}_A$ and the fringe deviation graphs obtained with the Gouy diffusiometer. These procedures are applied to recently reported data for free diffusion in aqueous solutions of mixtures of (1) LiCl and KCl and (2) LiCl and NaCl. New values for the four diffusion coefficients of each system are reported and compared with those obtained using the previous methods of calculation.

In general, the diffusion of either solute in a solution containing three components depends on the concentration gradients of both solutes, four diffusion coefficients being required to describe the system. This interaction or coupling of flows is conveniently represented by a modified form[2,3] of Onsager's[4] phenomenological flow equations. Data illustrating such interaction in two electrolyte systems have recently[3] been obtained using the Gouy diffusiometer.

In recent papers two procedures were developed for computing the four diffusion coefficients, but they are subject to certain limitations. A general method[2] utilizing reduced second and fourth moments, $\mathfrak{D}_{2m}$ and $\mathfrak{D}^2_{4m}$, of the refractive index gradient curves from two experiments is limited because of experimental inaccuracy in the reduced fourth moments. A second procedure[3] in which values of the diffusion coefficients are selected to reproduce best the observed fringe deviation graphs and reduced height–area ratios, $\mathfrak{D}_A$, of at least two experiments gives good results when it is applicable; however, it is based on series expansions for the concentration curves which are applicable only when one cross-term diffusion coefficient is sufficiently small.

Using the exact solutions derived below for the solute concentration distributions in free diffusion, new procedures are devised for calculating the four diffusion coefficients. The general method utilizing values of $\mathfrak{D}_A$ and $\mathfrak{D}_{2m}$ should be more accurate than the earlier procedure utilizing $\mathfrak{D}_{2m}$ and $\mathfrak{D}^2_{4m}$, because $\mathfrak{D}_A$ can be measured much more accurately than $\mathfrak{D}^2_{4m}$. The new second method, which depends primarily on values of $\mathfrak{D}_A$ and the fringe deviation graphs, has the advantage that neither cross-term diffusion coefficient need be small.

Theory

Basic Equations.—The equations for one-dimensional diffusion in a three-component system are written[5]

$$\frac{\partial C_1}{\partial t} = D_{11}\frac{\partial^2 C_1}{\partial x^2} + D_{12}\frac{\partial^2 C_2}{\partial x^2} \qquad (1)$$

$$\frac{\partial C_2}{\partial t} = D_{21}\frac{\partial^2 C_1}{\partial x^2} + D_{22}\frac{\partial^2 C_2}{\partial x^2} \qquad (2)$$

in which solute concentrations C_1 and C_2 are functions of position x and time t, D_{11} and D_{22} are the main diffusion coefficients, and D_{12} and D_{21} are the cross-term diffusion coefficients. For free diffu-

(1) On leave from the Department of Fisheries, Faculty of Agriculture, Kyoto University, Maizuru, Japan.

(2) R. L. Baldwin, P. J. Dunlop and L. J. Gosting, THIS JOURNAL, **77**, 5235 (1955).

(3) P. J. Dunlop and L. J. Gosting, *ibid.*, **77**, 5238 (1955).

(4) L. Onsager, *Ann. N. Y. Acad. Sci.*, **46**, 241 (1945).

(5) These equations are obtained by substituting the flow equations 1 and 2 of ref. 3 into the continuity equations. It is here assumed that the diffusion coefficients are all independent of concentration and that no volume change occurs on mixing. These conditions may be approached experimentally by making the concentration differences across the initial boundary sufficiently small. The reader is referred to refs. 2 and 3 for a more detailed description of the flow equations and the conditions under which they are valid.